UNITED STATES PATENT AND TRADEMARK OFFICE

UNITED STATES DEPARTMENT OF COMMERCE
United States Patent and Trademark Office
Address: COMMISSIONER FOR PATENTS
P.O. Box 1450
Alexandria, Virginia 22313-1450
www.uspto.gov

| APPLICATION NO. | FILING DATE | FIRST NAMED INVENTOR | ATTORNEY DOCKET NO. | CONFIRMATION NO. |
|---|---|---|---|---|
| 10/665,283 | 09/22/2003 | Renaud Derand | 243071US0 | 6920 |

22850 7590 10/20/2006

C. IRVIN MCCLELLAND
OBLON, SPIVAK, MCCLELLAND, MAIER & NEUSTADT, P.C.
1940 DUKE STREET
ALEXANDRIA, VA 22314

| EXAMINER | |
|---|---|
| DUNSTON, JENNIFER ANN | |
| **ART UNIT** | **PAPER NUMBER** |
| 1636 | |

DATE MAILED: 10/20/2006

Please find below and/or attached an Office communication concerning this application or proceeding.

| Office Action Summary | Application No. | Applicant(s) | |
|---|---|---|---|
| | 10/665,283 | DERAND ET AL. | |
| | Examiner | Art Unit | |
| | Jennifer Dunston | 1636 | |

*-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --*

**Period for Reply**

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

**Status**

1)☒ Responsive to communication(s) filed on *27 July 2006*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

**Disposition of Claims**

4)☒ Claim(s) *1,3-8,30,32-39,42 and 44-46* is/are pending in the application.

4a) Of the above claim(s) *30,32,33,36-39,42 and 44* is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *1,3,4,34,35,45 and 46* is/are rejected.

7)☒ Claim(s) *5-8* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

**Application Papers**

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

**Priority under 35 U.S.C. § 119**

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

**Attachment(s)**

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☒ Other: *Exhibits A-D*.

## DETAILED ACTION

### *Continued Examination Under 37 CFR 1.114*

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 6/13/2006 has been entered.

Receipt is acknowledged of an amendment, filed 6/13/2006, in which claims 2, 9-29, 31, 40-41 and 43 were canceled; and claims 1, 5-8, 36-39, 42 and 44 were amended. Currently, claims 1, 3-8, 30, 32-39, 42 and 44-46 are pending.

Any rejection of record in the previous office actions not addressed herein is withdrawn. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

### *Election/Restrictions*

Applicant elected Group I with traverse in the reply filed on 2/10/2005. Applicant also elected sub-species type (a) spacer, sub-species type (b) MRP1, and sub-species type (c) Kir6.2. The amended claims recite sufficiently few species to allow examination of all claims readable upon Group I.

Claims 30, 32, 33, 36-39, 42 and 44 are withdrawn from further consideration pursuant to 37 CFR 1.142(b), as being drawn to a nonelected invention, there being no allowable generic or

linking claim. Applicant timely traversed the restriction (election) requirement in the reply filed on 2/10/2005.

Currently, claims 1, 3-8, 34, 35, 45 and 46 are under consideration.

***Sequence Compliance***

This application contains sequence disclosures that are encompassed by the definitions for nucleotide and/or amino acid sequences set forth in 37 CFR 1.821(a)(1) and (a)(2). However, this application fails to comply with the requirements of 37 CFR 1.821 through 1.825 for the reason(s) set forth below.

Figure 4 contains the amino acid sequence FCYENE that is not referred to by the use of a sequence identifier. Where the description or claims of a patent application discuss a sequence that is set forth in the Sequence Listing, reference must be made to the sequence by use of the sequence identifier, preceded by "SEQ ID NO: " in the text of the description or claims, even if the sequence is also embedded in the text of the description or claims of the patent application.

In response to this office action, Applicant must comply with the sequence rules, 37 CFR 1.821 - 1.825. It would be remedial to amend the brief description of Figure 4 to indicate that the amino acid sequence FCYENE is SEQ ID NO: 25. The nature of the non-compliance did not preclude an examination of the elected invention on the merits, the results of which are presented below.

***Response to Arguments - Claim Objections***

The objection of claim25 is moot in view of Applicant's cancellation of the claim.

***Claim Rejections - 35 USC § 112***

Claims 1, 3, 4, 34, 35, 45 and 46 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. **This is a new matter rejection.**

Claim 1 is drawn to a hybrid protein comprising an ABC transporter membrane protein comprising a ligand-binding portion of MRP1, a spacer, an ATP-sensitive potassium ion channel portion of Kir6.2, and, optionally, a tag, wherein said spacer is between said ABC transporter membrane protein and said ATP-sensitive potassium channel, and wherein said membrane protein, spacer, and potassium channel are functionally coupled so that ligand binding to the ABC transporter membrane protein transduces a signal to the potassium channel that produces an electrical signal. The amendment to claim 1 to recite "ligand-binding portion" and "ion channel portion" is a departure from the specification as originally filed. Claims 3, 4, 34, 35, 45 and 46 depend from claim 1 and do not further limit these portions of the claimed protein.

Applicant has not pointed to portions of the specification that provide support. The term "portion" lacks antecedent basis in the instant specification. The instant specification does not describe the "ligand-binding portion" or "ion channel portion" of any protein. The specification envisions the fusion of an ABC transporter protein to an ion channel. The figures depict this fusion as the fusion of whole proteins (e.g. Figures 3 and 4). The working examples teach the

fusion of the full-length MRP1 to a Kir6.2 coding sequence (See Exhibits A-D). SEQ ID NO: 1 contains a full-length Kir6.2 coding sequence. SEQ ID NO: 4 contains a Kir6.2 coding sequence with a deletion of the 36 C-terminal residues. SEQ ID NO: 6 contains a Kir6.2 coding sequence with a KR370AA mutation. SEQ ID NO: 8 contains a full-length Kir6.2 coding sequence with an HA tag. Thus, the specification does not provide support for fusion proteins comprising a "ligand binding portion" or an "ion channel portion." Accordingly, the specification does not provide support for proteins comprising a "ligand-binding portion of MRP1." The only fragment of Kir6.2 described in the instant specification is the Kir6.2Δ36, which does not provide adequate support for the genus of proteins comprising an "ion channel portion of Kir6.2."

Accordingly, the amendment is a departure form the specification and claims as originally filed.

Claims 1, 3, 4, 34, 35, 45 and 46 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a hybrid protein consisting essentially of an MRP1 membrane protein, a spacer, and a Kir6.2 potassium channel, wherein said spacer is between said MRP2 membrane protein and said Kir6.2 potassium channel, does not reasonably provide enablement for a hybrid protein comprising only a ligand-binding portion of MRP1 and an ion channel portion of Kir6.2. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims. This is a new rejection, necessitated by the amendment filed 6/13/2006.

Enablement is considered in view of the Wands factors (MPEP 2164.01(A)). These include: nature of the invention, breadth of the claims, guidance of the specification, the existence of working examples, state of the art, predictability of the art and the amount of experimentation necessary. All of the Wands factors have been considered with regard to the instant claims, with the most relevant factors discussed below.

*Nature of the invention:* Claim 1 is drawn to a hybrid protein comprising an ABC transporter membrane protein comprising a ligand-binding portion of MRP1, a spacer, an ATP-sensitive potassium ion channel portion of Kir6.2, and, optionally, a tag, wherein said spacer is between said ABC transporter membrane protein and said ATP-sensitive potassium channel, and wherein said membrane protein, spacer, and potassium channel are functionally coupled so that ligand binding to the ABC transporter membrane protein transduces a signal to the potassium channel that produces an electrical signal. The amendment to claim 1 to recite "ligand-binding portion" and "ion channel portion" is a departure from the specification as originally filed. Claims 3, 4, 34, 35, 45 and 46 depend from claim 1 and do not further limit these portions of the claimed protein.

*Breadth of the claims:* The claims are broad in scope in that encompass proteins comprising any ligand-binding portion of an MRP1 protein and any ion channel portion of a Kir6.2 protein.

*Guidance of the specification and existence of working examples:* The specification envisions the use of the hybrid proteins as electrical sensors of membrane protein (e.g. receptor or transporter) activity such that the receptor or transporter occupancy by a ligand is transferred to the ion channel and transduced into an electrical signal that is detected by standard

electrophysiological techniques (e.g. page 1, lines 3-13; page 3, lines 1-5). The specification envisions the use of membrane proteins such as receptors, active transporters and passive transporters such as neurotransmitter receptors, hormone receptors, drug receptors, olfactive receptor, and heavy metal transporters (e.g. page 3, lines 24-28). Regarding the ion channel, the specification envisions the use of channels which have one or several of the following properties: they are coupled with a receptor/transporter in a physiological manner, they are encoded by a very small gene and easily handled by molecular biology, their gating behavior is straightforward and they are regulated and blocked by a simple ligand, which allows testing of the hybrid protein by simple electrophysiological assays (e.g. page 4, lines 1-7).

The working examples teach the fusion of the full-length MRP1 to a Kir6.2 coding sequence (See Exhibits A-D). SEQ ID NO: 1 contains a full-length Kir6.2 coding sequence. SEQ ID NO: 4 contains a Kir6.2 coding sequence with a deletion of the 36 C-terminal residues. SEQ ID NO: 6 contains a Kir6.2 coding sequence with a KR370AA mutation. SEQ ID NO: 8 contains a full-length Kir6.2 coding sequence with an HA tag. Thus, the specification does not teach fusion proteins comprising a "ligand binding portion" or an "ion channel portion." The only fragment or portion of Kir6.2 used to make a fusion protein is the Kir6.2Δ36, which does not provide adequate support for the genus of proteins comprising an "ion channel portion of Kir6.2."

The specification provides little or no guidance with regard to portions of MRP1 that can be functionally coupled to portions of Kir6.2.

*Predictability and state of the art:* In order to combine portions of MRP1 and Kir6.2 to make the claimed hybrid protein, one must be able to predict the ligand-binding portions

of MRP1 that can be functionally coupled to ion channel portions of Kir6.2. The prior art teaches that the prediction of protein conformation based upon the primary sequence is a highly unpredictable venture (Berendsen, Science, Vol. 282, pages 642-643, 1998). Lake (Nature, Vol. 409, page 558, 2001) acknowledges that computer structure prediction or threading cannot take the place of experiments. Mikhailov et al (The EMBO Journal, Vol. 24, pages 4166-4175, 2005) teach that Kir6.2 and SUR1, the naturally coupled combination disclosed in the instant specification, physically interact within both the transmembrane and cytosolic domains, which may both regulate the opening and closing of the pore (e.g. page 4171, paragraph bridging columns). Further, Mikhailov et al teach that the Kir6.2-SUR1 structure is one of the few obtained for large mammalian plasma membrane protein complexes, and is unusual in being derived from a recombinant fusion protein (e.g. page 4172, right column, 1st full paragraph; Figure 1A). Given the complex structure taught by the post filing art (for example, Figure 6 of Mikhailov et al) and the unpredictable nature of protein structure prediction, it would have been an extremely unpredictable venture at the time the invention was made to predict which portions of MRP1 and which portions of Kir6.2 could be functionally coupled.

*Amount of experimentation necessary:* The quantity of experimentation required to carry out the claimed invention is very large, as the skilled artisan could not rely upon the prior art or instant specification to select the portions of MRP1 and Kir6.2 that can be functionally coupled within a fusion protein. A large amount of experimentation requiring a large amount of inventive effort would be required to make and use proteins commensurate in scope with the claims.

In view of the breadth of the claims and the lack of guidance provided by the specification as well as the unpredictability of the art, the skilled artisan would have required an undue amount of experimentation to make and/or use the claimed invention. Therefore, claims 1, 3, 4, 34, 35, 45 and 46 are not considered to be fully enabled by the instant specification.

Claims 1, 3, 4, 34, 35, 45 and 46 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the written description requirement. The claim(s) contains subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventor(s), at the time the application was filed, had possession of the claimed invention. This is a new rejection, necessitated by the amendment filed 6/13/2006.

Claim 1 is drawn to a hybrid protein comprising an ABC transporter membrane protein comprising a ligand-binding portion of MRP1, a spacer, an ATP-sensitive potassium ion channel portion of Kir6.2, and, optionally, a tag, wherein said spacer is between said ABC transporter membrane protein and said ATP-sensitive potassium channel, and wherein said membrane protein, spacer, and potassium channel are functionally coupled so that ligand binding to the ABC transporter membrane protein transduces a signal to the potassium channel that produces an electrical signal. The amendment to claim 1 to recite "ligand-binding portion" and "ion channel portion" is a departure from the specification as originally filed. Claims 3, 4, 34, 35, 45 and 46 depend from claim 1 and do not further limit these portions of the claimed protein. Thus, the claims are drawn to a genus of hybrid proteins comprising a genus of fragments of MRP1 containing a ligand-binding portion, and a genus of fragment of Kir6.2 comprising an ion channel portion.

To provide adequate written description and evidence of possession of a claimed genus, the specification must provide sufficient distinguishing identifying characteristics of the genus. The factors to be considered include disclosure of a complete or partial structure, physical and/or chemical properties, functional characteristics, structure/function correlation, and any combination thereof. The specification envisions the use of the hybrid proteins as electrical sensors of membrane protein (e.g. receptor or transporter) activity such that the receptor or transporter occupancy by a ligand is transferred to the ion channel and transduced into an electrical signal that is detected by standard electrophysiological techniques (e.g. page 1, lines 3-13; page 3, lines 1-5). The specification envisions the use of membrane proteins such as receptors, active transporters and passive transporters such as neurotransmitter receptors, hormone receptors, drug receptors, olfactive receptor, and heavy metal transporters (e.g. page 3, lines 24-28). Regarding the ion channel, the specification envisions the use of channels which have one or several of the following properties: they are coupled with a receptor/transporter in a physiological manner, they are encoded by a very small gene and easily handled by molecular biology, their gating behavior is straightforward and they are regulated and blocked by a simple ligand, which allows testing of the hybrid protein by simple electrophysiological assays (e.g. page 4, lines 1-7). Furthermore, the specification envisions the use of functional derivatives of membrane proteins and ion channels (e.g. pages 4-5). The specification describes the fusion of the full-length MRP1 to a Kir6.2 coding sequence (See Exhibits A-D). SEQ ID NO: 1 contains a full-length Kir6.2 coding sequence. SEQ ID NO: 4 contains a Kir6.2 coding sequence with a deletion of the 36 C-terminal residues. SEQ ID NO: 6 contains a Kir6.2 coding sequence with a KR370AA mutation. SEQ ID NO: 8 contains a full-length Kir6.2 coding sequence with an HA

tag. No description is provided of the fusion of any fragment of MRP1 to Kir6.2. No description is provided of the genus of fragments of MRP1 and Kir6.2 that are capable of being functionally coupled. A representative number of species of membrane proteins that meet the claim limitations have not been disclosed, and no structural/functional relationship is provided to allow one of skill in the art to envision a representative number of members of this genus.

Even if one accepts that the examples described in the specification meet the claim limitations of the rejected claims with regard to structure and function, the examples are only representative of a few hybrid proteins. The results are not necessarily predictive of other hybrid proteins comprising a ligand-binding portion of MRP1 and an ion channel portion of Kir6.2. Thus, it is impossible for one to extrapolate from the few examples described herein those hybrid proteins that would necessarily meet the structural/functional characteristics of the rejected claims.

The prior art does not appear to offset the deficiencies of the instant specification in that the art of record does not describe a set of hybrid proteins that provide sufficient structural/functional information for one of skill in the art to envision other members of the genus. The post filing art teaches a complex structure for a Kir6.2-SUR1 fusion protein, with contacts in the transmembrane domain and cytosolic domain that are likely to regulate ligand gating of the channel (Mikhailov et al, The EMBO Journal, Vol. 24, No. 23, pages 4166-4175, 2005; e.g. page 4171, paragraph bridging columns, Figure 6). The general knowledge and level of skill in the art do not supplement the omitted description because specific, not general, guidance is what is needed.

*Vas-Cath Inc. v. Mahurkar*, 19USPQ2d 1111, clearly states, "applicant must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of *the invention.* The invention is, for purposes of the 'written description' inquiry, *whatever is now claimed.*" (See page 1117.) The specification does not "clearly allow persons of ordinary skill in the art to recognize that [he or she] invented what is now is claimed." (See *Vas-Cath* at page 1116). As discussed above, the skilled artisan cannot envision the detailed chemical structure of the encompassed genus of hybrid proteins, and therefore conception is not achieved until reduction to practice has occurred, regardless of the complexity or simplicity of the method of isolation or identification. Adequate written description requires more than a mere statement that it is part of the invention and reference to a potential method of isolating it. The compound itself is required. See *Fiers v. Revel*, 25USPQ2d 1601 at 1606 (CAFC 1993) and *Amgen Inc. v. Chugai Pharmaceutical Co. Ltd.*, 18USPQ2d 1016.

One cannot describe what one has not conceived. See *Fiddes v. Baird*, 30 USPQ2d 1481 at 1483. In *Fiddes*, claims directed to mammalian FGFs were found to be unpatentable due to lack of written description for that broad class. The specification provided only the bovine sequence.

Given the very large genus of hybrid proteins encompassed by the rejected claims, and given the limited description provided by the prior art and specification, the skilled artisan would not have been able to envision a sufficient number of specific embodiments that meet the limitations of the claims to describe the broadly claimed genus. The disclosed sequences of SEQ ID NOS: 1, 4, 6 and 8 alone are insufficient to describe the genus. Therefore, the skilled artisan

would have reasonably concluded applicants were not in possession of a representative number of species to describe the claimed genus for claims 1, 3, 4, 34, 35, 45 and 46.

***Response to Arguments - 35 USC § 112***

Applicant's arguments with respect to claims 1, 3, 13, 15, 24, 34, 35, 45 and 46 have been considered but are moot in view of the new ground(s) of rejection.

***Conclusion***

Claims 5-8 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form without including the new matter introduced into independent claim 1.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Jennifer Dunston whose telephone number is 571-272-2916. The examiner can normally be reached on M-F, 9 am to 5 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Remy Yucel can be reached on 571-272-0781. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

Jennifer Dunston, Ph.D.
Examiner
Art Unit 1636

jad

CELINE QIAN, PH.D.
PRIMARY EXAMINER

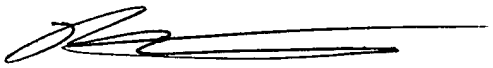

Exhibit A: SEQ ID NO: 1

```
RESULT 1
AAW57486
ID   AAW57486 standard; protein; 1531 AA.
XX
AC   AAW57486;
XX
DT   14-AUG-1998  (first entry)
XX
DE   Human MRP variant ltPgpA (Lei/PgpA).
XX
KW   Multidrug resistance-associated protein; MRP; tumour; human; variant;
KW   multidrug resistance; MDR; leismania P-glycoprotein; ltPgpA; Lei/PgpA.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   Misc-difference 685
FT                   /label= L685S
FT                   /note= "wild-type Leu is replaced by Ser"
FT   Misc-difference 1282
FT                   /label= R1282A
FT                   /note= "wild-type Arg is replaced by Ala"
XX
PN   US5766880-A.
XX
PD   16-JUN-1998.
XX
PF   05-JUN-1995;   95US-00463092.
XX
PR   27-OCT-1992;   92US-00966923.
PR   08-MAR-1993;   93US-00029340.
PR   26-OCT-1993;   93US-00141893.
PR   20-MAR-1995;   95US-00407207.
XX
PA   (TOOH ) UNIV QUEENS KINGSTON.
XX
PI   Cole SP,  Deeley RG;
XX
DR   WPI; 1998-361687/31.
DR   N-PSDB; AAV31498.
XX
PT   DNA encoding protein associated with multi-drug resistance - useful for
PT   as probe for identifying multi-drug resistant tumour cells.
XX
PS   Claim 1; Col 67-78; 82pp; English.
XX
CC   This represents a variant of the human multidrug resistance-associated
CC   protein (MRP). This natural variant is a leismania P-glycoprotein related
CC   molecule ltPgpA (Lei/PgpA). The human and murine MRP nucleic acid
CC   molecules can be used as probes for identifying multidrug resistant
CC   tumour cells by hybridisation to mRNA from tumour cells. The antisense
CC   nucleic acid can be used to reverse multidrug resistance (MDR). A
CC   recombinant expression vector containing the MRP nucleic acid molecules
CC   operatively linked to at least one regulatory sequence can be used to
CC   transform a host cell to produce a recombinant MDR-associated protein
XX
SQ   Sequence 1531 AA;

  Query Match             79.4%;  Score 7860;  DB 2;  Length 1531;
  Best Local Similarity   100.0%;  Pred. No. 0;
  Matches 1531;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;
```

```
Qy          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60

Qy         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120

Qy        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180

Qy        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240

Qy        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300

Qy        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360

Qy        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420

Qy        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480

Qy        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540

Qy        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600

Qy        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660

Qy        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720

Qy        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780

Qy        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840

Qy        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
```

```
Qy       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960

Qy       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020

Qy      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080

Qy      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140

Qy      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200

Qy      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260

Qy      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320

Qy      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380

Qy      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440

Qy      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500

Qy      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
            ||||||||||||||||||||||||||||||||
Db      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
```

Exhibit B: SEQ ID NO:4

```
RESULT 5
AAW57486
ID   AAW57486 standard; protein; 1531 AA.
XX
AC   AAW57486;
XX
DT   14-AUG-1998  (first entry)
XX
DE   Human MRP variant ltPgpA (Lei/PgpA).
XX
KW   Multidrug resistance-associated protein; MRP; tumour; human; variant;
KW   multidrug resistance; MDR; leismania P-glycoprotein; ltPgpA; Lei/PgpA.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   Misc-difference 685
FT                   /label= L685S
FT                   /note= "wild-type Leu is replaced by Ser"
FT   Misc-difference 1282
FT                   /label= R1282A
FT                   /note= "wild-type Arg is replaced by Ala"
XX
PN   US5766880-A.
XX
PD   16-JUN-1998.
XX
PF   05-JUN-1995;   95US-00463092.
XX
PR   27-OCT-1992;   92US-00966923.
PR   08-MAR-1993;   93US-00029340.
PR   26-OCT-1993;   93US-00141893.
PR   20-MAR-1995;   95US-00407207.
XX
PA   (TOOH ) UNIV QUEENS KINGSTON.
XX
PI   Cole SP,  Deeley RG;
XX
DR   WPI; 1998-361687/31.
DR   N-PSDB; AAV31498.
XX
PT   DNA encoding protein associated with multi-drug resistance - useful for
PT   as probe for identifying multi-drug resistant tumour cells.
XX
PS   Claim 1; Col 67-78; 82pp; English.
XX
CC   This represents a variant of the human multidrug resistance-associated
CC   protein (MRP). This natural variant is a leismania P-glycoprotein related
CC   molecule ltPgpA (Lei/PgpA). The human and murine MRP nucleic acid
CC   molecules can be used as probes for identifying multidrug resistant
CC   tumour cells by hybridisation to mRNA from tumour cells. The antisense
CC   nucleic acid can be used to reverse multidrug resistance (MDR). A
CC   recombinant expression vector containing the MRP nucleic acid molecules
CC   operatively linked to at least one regulatory sequence can be used to
CC   transform a host cell to produce a recombinant MDR-associated protein
XX
SQ   Sequence 1531 AA;

  Query Match             80.7%;  Score 7860;  DB 2;  Length 1531;
  Best Local Similarity   100.0%;  Pred. No. 0;
  Matches 1531;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;
```

```
Qy          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60

Qy         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120

Qy        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180

Qy        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240

Qy        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300

Qy        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360

Qy        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420

Qy        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480

Qy        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540

Qy        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600

Qy        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660

Qy        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720

Qy        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780

Qy        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840

Qy        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
```

```
Qy       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960

Qy       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020

Qy      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080

Qy      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140

Qy      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200

Qy      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260

Qy      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320

Qy      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380

Qy      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440

Qy      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500
            ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500

Qy      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
            |||||||||||||||||||||||||||||||
Db      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
```

Exhibit C: SEQ ID NO:6

```
RESULT 5
AAW57486
ID   AAW57486 standard; protein; 1531 AA.
XX
AC   AAW57486;
XX
DT   14-AUG-1998  (first entry)
XX
DE   Human MRP variant ltPgpA (Lei/PgpA).
XX
KW   Multidrug resistance-associated protein; MRP; tumour; human; variant;
KW   multidrug resistance; MDR; leismania P-glycoprotein; ltPgpA; Lei/PgpA.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   Misc-difference 685
FT                   /label= L685S
FT                   /note= "wild-type Leu is replaced by Ser"
FT   Misc-difference 1282
FT                   /label= R1282A
FT                   /note= "wild-type Arg is replaced by Ala"
XX
PN   US5766880-A.
XX
PD   16-JUN-1998.
XX
PF   05-JUN-1995;   95US-00463092.
XX
PR   27-OCT-1992;   92US-00966923.
PR   08-MAR-1993;   93US-00029340.
PR   26-OCT-1993;   93US-00141893.
PR   20-MAR-1995;   95US-00407207.
XX
PA   (TOOH ) UNIV QUEENS KINGSTON.
XX
PI   Cole SP,  Deeley RG;
XX
DR   WPI; 1998-361687/31.
DR   N-PSDB; AAV31498.
XX
PT   DNA encoding protein associated with multi-drug resistance - useful for
PT   as probe for identifying multi-drug resistant tumour cells.
XX
PS   Claim 1; Col 67-78; 82pp; English.
XX
CC   This represents a variant of the human multidrug resistance-associated
CC   protein (MRP). This natural variant is a leismania P-glycoprotein related
CC   molecule ltPgpA (Lei/PgpA). The human and murine MRP nucleic acid
CC   molecules can be used as probes for identifying multidrug resistant
CC   tumour cells by hybridisation to mRNA from tumour cells. The antisense
CC   nucleic acid can be used to reverse multidrug resistance (MDR). A
CC   recombinant expression vector containing the MRP nucleic acid molecules
CC   operatively linked to at least one regulatory sequence can be used to
CC   transform a host cell to produce a recombinant MDR-associated protein
XX
SQ   Sequence 1531 AA;

  Query Match             79.4%;  Score 7860;  DB 2;  Length 1531;
  Best Local Similarity   100.0%;  Pred. No. 0;
  Matches 1531;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;
```

```
Qy          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60

Qy         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120

Qy        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180

Qy        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240

Qy        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300

Qy        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360

Qy        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420

Qy        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480

Qy        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540

Qy        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600

Qy        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660

Qy        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720

Qy        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780

Qy        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840

Qy        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
```

```
Qy       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960

Qy       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020

Qy      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080

Qy      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140

Qy      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200

Qy      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260

Qy      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320

Qy      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380

Qy      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440

Qy      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500

Qy      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
             |||||||||||||||||||||||||||||||
Db      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
```

Exhibit D: SEQ ID NO:8

```
RESULT 1
AAW57486
ID   AAW57486 standard; protein; 1531 AA.
XX
AC   AAW57486;
XX
DT   14-AUG-1998  (first entry)
XX
DE   Human MRP variant ltPgpA (Lei/PgpA).
XX
KW   Multidrug resistance-associated protein; MRP; tumour; human; variant;
KW   multidrug resistance; MDR; leismania P-glycoprotein; ltPgpA; Lei/PgpA.
XX
OS   Homo sapiens.
XX
FH   Key             Location/Qualifiers
FT   Misc-difference 685
FT                   /label= L685S
FT                   /note= "wild-type Leu is replaced by Ser"
FT   Misc-difference 1282
FT                   /label= R1282A
FT                   /note= "wild-type Arg is replaced by Ala"
XX
PN   US5766880-A.
XX
PD   16-JUN-1998.
XX
PF   05-JUN-1995;   95US-00463092.
XX
PR   27-OCT-1992;   92US-00966923.
PR   08-MAR-1993;   93US-00029340.
PR   26-OCT-1993;   93US-00141893.
PR   20-MAR-1995;   95US-00407207.
XX
PA   (TOOH ) UNIV QUEENS KINGSTON.
XX
PI   Cole SP,  Deeley RG;
XX
DR   WPI; 1998-361687/31.
DR   N-PSDB; AAV31498.
XX
PT   DNA encoding protein associated with multi-drug resistance - useful for
PT   as probe for identifying multi-drug resistant tumour cells.
XX
PS   Claim 1; Col 67-78; 82pp; English.
XX
CC   This represents a variant of the human multidrug resistance-associated
CC   protein (MRP). This natural variant is a leismania P-glycoprotein related
CC   molecule ltPgpA (Lei/PgpA). The human and murine MRP nucleic acid
CC   molecules can be used as probes for identifying multidrug resistant
CC   tumour cells by hybridisation to mRNA from tumour cells. The antisense
CC   nucleic acid can be used to reverse multidrug resistance (MDR). A
CC   recombinant expression vector containing the MRP nucleic acid molecules
CC   operatively linked to at least one regulatory sequence can be used to
CC   transform a host cell to produce a recombinant MDR-associated protein
XX
SQ   Sequence 1531 AA;

  Query Match             78.5%;  Score 7860;  DB 2;  Length 1531;
  Best Local Similarity   100.0%;  Pred. No. 0;
  Matches 1531;  Conservative    0;  Mismatches    0;  Indels    0;  Gaps    0;
```

```
Qy          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MALRGFCSADGSDPLWDWNVTWNTSNPDFTKCFQNTVLVWVPCFYLWACFPFYFLYLSRH 60

Qy         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 DRGYIQMTPLNKTKTALGFLLWIVCWADLFYSFWERSRGIFLAPVFLVSPTLLGITTLLA 120

Qy        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        121 TFLIQLERRKGVQSSGIMLTFWLVALVCALAILRSKIMTALKEDAQVDLFRDITFYVYFS 180

Qy        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        181 LLLIQLVLSCFSDRSPLFSETIHDPNPCPESSASFLSRITFWWITGLIVRGYRQPLEGSD 240

Qy        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        241 LWSLNKEDTSEQVVPVLVKNWKKECAKTRKQPVKVVYSSKDPAQPKESSKVDANEEVEAL 300

Qy        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        301 IVKSPQKEWNPSLFKVLYKTFGPYFLMSFFFKAIHDLMMFSGPQILKLLIKFVNDTKAPD 360

Qy        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        361 WQGYFYTVLLFVTACLQTLVLHQYFHICFVSGMRIKTAVIGAVYRKALVITNSARKSSTV 420

Qy        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        421 GEIVNLMSVDAQRFMDLATYINMIWSAPLQVILALYLLWLNLGPSVLAGVAVMVLMVPVN 480

Qy        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        481 AVMAMKTKTYQVAHMKSKDNRIKLMNEILNGIKVLKLYAWELAFKDKVLAIRQEELKVLK 540

Qy        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        541 KSAYLSAVGTFTWVCTPFLVALCTFAVYVTIDENNILDAQTAFVSLALFNILRFPLNILP 600

Qy        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        601 MVISSIVQASVSLKRLRIFLSHEELEPDSIERRPVKDGGGTNSITVRNATFTWARSDPPT 660

Qy        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        661 LNGITFSIPEGALVAVVGQVGCGKSSLLSALLAEMDKVEGHVAIKGSVAYVPQQAWIQND 720

Qy        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        721 SLRENILFGCQLEEPYYRSVIQACALLPDLEILPSGDRTEIGEKGVNLSGGQKQRVSLAR 780

Qy        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        781 AVYSNADIYLFDDPLSAVDAHVGKHIFENVIGPKGMLKNKTRILVTHSMSYLPQVDVIIV 840

Qy        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db        841 MSGGKISEMGSYQELLARDGAFAEFLRTYASTEQEQDAEENGVTGVSGPGKEAKQMENGM 900
```

```
Qy       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       901 LVTDSAGKQLQRQLSSSSSYSGDISRHHNSTAELQKAEAKKEETWKLMEADKAQTGQVKL 960

Qy       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       961 SVYWDYMKAIGLFISFLSIFLFMCNHVSALASNYWLSLWTDDPIVNGTQEHTKVRLSVYG 1020

Qy      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1021 ALGISQGIAVFGYSMAVSIGGILASRCLHVDLLHSILRSPMSFFERTPSGNLVNRFSKEL 1080

Qy      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1081 DTVDSMIPEVIKMFMGSLFNVIGACIVILLATPIAAIIIPPLGLIYFFVQRFYVASSRQL 1140

Qy      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1141 KRLESVSRSPVYSHFNETLLGVSVIRAFEEQERFIHQSDLKVDENQKAYYPSIVANRWLA 1200

Qy      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1201 VRLECVGNCIVLFAALFAVISRHSLSAGLVGLSVSYSLQVTTYLNWLVRMSSEMETNIVA 1260

Qy      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1261 VERLKEYSETEKEAPWQIQETAPPSSWPQVGRVEFRNYCLRYREDLDFVLRHINVTINGG 1320

Qy      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1321 EKVGIVGRTGAGKSSLTLGLFRINESAEGEIIIDGINIAKIGLHDLRFKITIIPQDPVLF 1380

Qy      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1381 SGSLRMNLDPFSQYSDEEVWTSLELAHLKDFVSALPDKLDHECAEGGENLSVGQRQLVCL 1440

Qy      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500
             ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db      1441 ARALLRKTKILVLDEATAAVDLETDDLIQSTIRTQFEDCTVLTIAHRLNTIMDYTRVIVL 1500

Qy      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
             |||||||||||||||||||||||||||||||
Db      1501 DKGEIQEYGAPSDLLQQRGLFYSMAKDAGLV 1531
```